K091053

Attachment D
510(k) SUMMARY

NOV 2 0 2009

CONTACT

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NAME OF DEVICE

Trade Name:	ProParaflu+™ Assay
Regulation Number:	21 CFR 866.3980
Classification Name:	Respiratory viral panel multiplex nucleic acid assay

PREDICATE DEVICE

K063765 – ID Tag Respiratory Virus Panel, Luminex Molecular Diagnostics

INTENDED USE

The ProParaflu+ Assay is a multiplex Real Time RT-PCR *in vitro* diagnostic test for the qualitative detection and discrimination of Parainfluenza 1 Virus, Parainfluenza 2 Virus and Parainfluenza 3 Virus (HPIV-1, HPIV-2 and HPIV-3) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of respiratory tract infections. This assay targets the conserved regions of the Hemagglutinin-Neuraminidase (HN) gene of HPIV-1, HPIV-2 and HPIV-3, respectively. The detection and discrimination of HPIV-1, HPIV-2 and HPIV-3 nucleic acids from symptomatic patients aid in the diagnosis of human respiratory tract parainfluenza infections if used in conjunction with other clinical and laboratory findings. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b Viruses.

Negative test results are presumptive and should be confirmed by cell culture. Negative results do not preclude Parainfluenza 1, 2 or 3 virus infections and should not be used as the sole basis for treatment or other management decisions.

PRODUCT DESCRIPTION

The ProParaflu+ Assay enables the detection and differentiation of Parainfluenza 1 Virus, Parainfluenza 2 Virus, Parainfluenza 3 Virus and an Internal Control (IC) nucleic acid. Nasopharyngeal swab specimens from symptomatic patients using a polyester, rayon or nylon tipped swab and place into viral transport medium. The IC is added to every sample prior to nucleic acid extraction to monitor for inhibitors present in the specimens.

Isolation and purification of nucleic acids is performed using the bioMérieux NucliSENS

easyMAG automated extractor and the Automated Magnetic Extraction Reagents or the Roche MagNA Pure LC Instrument and the MagNA Pure Total Nucleic Acid Isolation Kit.

The purified nucleic acids are added to the ProParaflu+ Supermix along with enzymes included in the ProParaflu+ Detection Kit. The ProParaflu+ Supermix contains oligonucleotide primers that are complementary to highly conserved regions of hemagglutinin neuraminidase gene for each human Parainfluenza type (1, 2 and 3). The probes are dual-labeled with a reporter dye attached to the 5'-end and a quencher dye attached to the 3'-end (see table below).

RT-PCR amplification is performed in a Cepheid SmartCycler® II instrument. During this process, the primers and probes anneal specifically to the template (if present) followed by primer extension and amplification. The ProParaflu+ Assay is based on Taqman chemistry, which utilizes the 5' – 3' exonuclease activity of the Taq polymerase to cleave the probe thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle, additional reporter dye molecules are cleaved from their respective probes, further increasing the fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification product present at that time. Fluorescent intensity is monitored during each PCR cycle by the real-time instrument. Results are analyzed and interpreted as presented by the software.

Analyte	Gene Targeted	Probe Fluorophore	Absorbance Peak	Emission Peak	Instrument Channel
Parainfluenza 1 Virus	Hemagglutinin neuraminidase	FAM	495 nm	520 nm	FAM
Parainfluenza 3 Virus	Hemagglutinin neuraminidase	Cal Orange 560	540 nm	561 nm	TET
Parainfluenza 2 Virus	Hemagglutinin neuraminidase	Cal Red 610	595 nm	615 nm	Texas Red
Internal Control	NA	Quasar 670	647 nm	667 nm	Cy5

SUBSTANTIAL EQUIVALENCE

Clinical Performance

The clinical performance of the ProParaflu+ Assay was established during a prospective study at 4 U.S. clinical laboratories during May 2008 – September 2009. Specimens used in the study represented excess nasopharyngeal (NP) swab specimens that were prospectively collected from symptomatic individuals suspected of respiratory infection, and were submitted for routine analysis. Demographic details for this patient population are summarized in the following table.

Gender and Age Demographic Detail for ProParaflu+ Prospective Study

Sex	Number of Subjects
Female	407 (47.5%)
Male	450 (52.5%)
Age (yrs)	
≤ 5 years	580 (67.7%)
6 - 21 years	168 (19.6%)
22 – 59 years	67 (7.8%)
≥ 60 years	42 (4.9%)

Performance of the ProParaflu+ Assay was compared to the reference method of cell culture (rapid or traditional) followed by direct fluorescent antibody (DFA) screening and HPIV type identification.

A total of 857 eligible NP swab samples were tested with the ProParaflu+ Assay and by culture across four clinical sites. Of the ProParaflu+ Assay run on all eligible specimens, 99.2% (852/857) of these specimens were successful on the first attempt. The remaining 5 gave "Unresolved" results on the first attempt. Unresolved results occur when the sample is negative for all three HPIVs and the Internal Control, indicating potentially PCR-inhibiting samples. Of the 5 "Unresolved" specimens on the first attempt, 60.0% (3/5) gave a valid result on the second attempt. The remaining 2 were "Unresolved" on the second attempt and are not included in the analysis below. Both samples were culture negative.

Discrepant analysis for samples where ProParaflu+ Assay and culture results were in disagreement was performed using RT-PCR with virus specific primers obtained from literature[8, 9] (and different from those used in ProParaflu+) followed by bi-directional sequencing.

Prospective Study

Parainfluenza 1 Comparison Results

		Culture/DFA			
		Positive	**Negative**	**Total**	
ProParaflu+ Assay	**Positive**	16	1[a]	**17**	Sensitivity 88.9% (67.2% - 96.9%) 95% CI
	Negative	2[b]	838	**840**	Specificity 99.9% (99.3% - 100.0%) 95% CI
	Total	**18**	**839**	**857**	

[a]One (1) sample positive for HPIV-1 by bi-directional sequence analysis.

[b]Two (2) samples negative for HPIV-1 by bi-directional sequence analysis. One sample positive for HPIV-3 by ProParaflu+ and bi-directional sequence analysis.

Parainfluenza 2 Comparison Results

		Culture/DFA			
		Positive	**Negative**	**Total**	
ProParaflu+ Assay	**Positive**	26	2[a]	**28**	Sensitivity 96.3% (81.7% - 99.3%) 95% CI
	Negative	1[b]	828	**829**	Specificity 99.8% (99.1% - 99.9%) 95% CI
	Total	**27**	**830**	**857**	

[a]Two (2) samples positive for HPIV-2 by bi-directional sequence analysis.

[b]One (1) sample negative for HPIV-2 by bi-directional sequence analysis.

Parainfluenza 3 Comparison Results

		Culture/DFA			
		Positive	**Negative**	**Total**	
ProParaflu+ Assay	**Positive**	36	8[a]	**44**	Sensitivity 97.3% (86.2% - 99.5%) 95% CI
	Negative	1[b]	812	**813**	Specificity 99.2% (98.1% - 99.5%) 95% CI
	Total	**37**	**820**	**857**	

[a]Seven (7) samples positive for HPIV-3 and one (1) sample negative for HPIV-3 by bi-directional sequence analysis.

[b]One (1) sample negative for HPIV-3 by bi-directional sequence analysis.

Retrospective Study

Due to a minimal number of HPIV-1 positive samples, a retrospective study was also conducted using a total of 91 frozen NP swab samples that had been previously tested by direct DFA. Demographic details for this patient population are summarized in the following table.

Gender and Age Demographic Detail for ProParaflu+ Retrospective Study

Sex	Number of Subjects
Female	40 (44.4%)
Male	50 (55.6%)
Age (yrs)	
≤ 5 years	81 (90.0%)
6 - 21 years	5 (5.6%)
22 – 59 years	2 (2.2%)
≥ 60 years	2 (2.2%)

Parainfluenza 1 Comparison Results

		DFA			
		Positive	**Negative**	**Total**	
ProParaflu+ Assay	**Positive**	24	0	**24**	Sensitivity 82.8% (65.4% - 92.4%) 95% CI
	Negative	5[a]	62	**67**	Specificity 100% (94.2% - 100%) 95% CI
	Total	**29**	**62**	**91**	

[a]Five (5) samples negative for HPIV-1 by bi-directional sequence analysis.

Reproducibility

The reproducibility of the ProParaflu+ Assay was evaluated at 3 laboratory sites. Reproducibility was assessed using a panel of 12 simulated samples that included medium positive, low positive (near the assay limit of detection, ≥95% positive), intermediate (1 log below the assay limit of detection), and high negative (below the assay limit of detection, <5% positive) samples. Panels and controls were tested at each site by 2 operators for 5 days. The overall percent agreement with the expected result for the ProParaflu+ Assay was 97.8%.

	Panel Member ID	HPIV-1 high negative[a]	HPIV-1 low positive	HPIV-1 medium positive	HPIV-2 high negative[a]	HPIV-2 low positive	HPIV-2 medium positive	HPIV-3 high negative[a]	HPIV-3 low positive	HPIV-3 medium positive	Para Extraction Control	Para RNA Control			Negative Control	Total % Agreement
												HPIV-1	HPIV-2	HPIV-3		
	Concentration	0.001 X LoD	2 X LoD	10X LoD	0.001 X LoD	2 X LoD	10X LoD	0.01 X LoD	2 X LoD	10X LoD	N/A	N/A			N/A	
Site 1	Agreement with Expected Result	10/10 100%	8/10 80%	9/9 100%	10/10 100%	9/9 100%	9/10 90%	10/10 100%	9/10 90%	10/10 100%	10/10 100%	10/10 100%			10/10 100%	**114/118 96.6%**
	Mean Ct Value	27.73	28.31	26.33	27.90	28.62	26.27	27.76	31.21	29.47	27.33	27.37	29.37	28.61	27.69	
	% CV	2.87	1.55	1.60	2.84	0.85	1.25	2.43	3.21	1.91	1.60	1.05	0.43	0.81	1.67	
Site 2	Agreement with Expected Result	8/10 80%	8/10 80%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%			10/10 100%	**116/120 96.7%**
	Mean Ct Value	28.59	28.47	26.12	28.83	28.91	26.61	28.30	31.64	29.51	27.56	23.86	26.09	25.23	28.68	
	% CV	1.36	1.72	1.26	2.80	1.31	1.83	1.18	2.17	2.66	2.47	1.48	1.12	0.92	1.15	
Site 3	Agreement with Expected Result	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%	10/10 100%			10/10 100%	**120/120 100%**
	Mean Ct Value	26.35	29.91	27.67	26.28	29.51	27.44	26.67	33.13	30.43	28.61	28.73	30.98	29.84	26.46	
	% CV	0.88	0.81	1.25	1.35	0.57	2.25	4.13	2.36	1.02	1.54	3.31	3.39	3.19	1.04	
	Total Agreement with Expected Result	28/30 93.3%	26/30 86.7%	29/29 100%	30/30 100%	29/29 100%	29/30 96.7%	30/30 100%	29/30 96.7%	30/30 100%	30/30 100%	30/30 100%			30/30 100%	**350/358 97.8%**
	95% CI	78.7% - 98.2%	70.3% - 94.7%	88.3% - 100%	88.6% - 100%	88.3% - 100%	83.3%- 99.4%	88.6% - 100%	83.3%- 99.4%	88.6%- 100%	88.6% - 100%	88.6% - 100%			88.6%- 100%	**95.6% - 98.9%**
	Overall Mean Ct Value	27.56	28.90	26.72	27.67	29.03	26.79	27.58	32.02	29.80	27.83	26.65	28.81	27.89	27.62	
	Overall % CV	3.88	2.87	2.95	4.55	1.57	2.54	3.68	3.61	2.42	2.75	8.13	7.49	7.39	3.54	

[a]Average Ct value for the Internal Control (IC)

An additional reproducibility study was performed to assess samples that were at an intermediate concentration, below the assay's LoD but above the "high negatives" tested during the original reproducibility study. The percent positive for the intermediate member across all sites was 56.7% for HPIV-1 (mean Ct = 35.1), 86.7% for HPIV-2 (mean Ct = 33.0), and 30.0% for HPIV-3 (mean Ct = 37.1). This result was expected as the intermediate concentration should be positive in the range of 5 - 95% as the samples were lower concentration than the LoD concentration (≥ 95% positive) and higher than the "high negative" concentration (< 5% positive).

	Panel Member ID	HPIV-1 intermediate	HPIV-2 intermediate	HPIV-3 intermediate	Para Extraction Control	Parainfluenza RNA Control			Negative Control[a]
						HPIV-1	HPIV-2	HPIV-3	
	Concentration	0.1 X LoD	0.1 X LoD	0.1 X LoD	N/A	N/A			N/A
Site 1	Agreement with Positive Result	4/10 40%	8/10 80%	1/10 10%	10/10 100%	10/10 100%			10/10* 100%
	Average Ct Value	35.5	33.3	36.9	27.9	28.8	30.4	29.5	28.4
	% CV	6.19	2.78	N/A	3.80	1.13	0.88	0.89	3.47
Site 2	Agreement with Positive Result	8/10 80%	10/10 100%	7/10 70%	10/10 100%	10/10 100%			10/10 100%
	Average Ct Value	34.4	32.2	37.3	27.5	29.0	30.6	29.9	27.7
	% CV	1.38	2.22	1.50	2.92	1.04	0.89	0.72	2.80
Site 3	Agreement with Positive Result	5/10 50%	8/10 80%	1/10 10%	10/10 100%	10/10 100%			10/10 100%
	Average Ct Value	35.9	33.7	35.8	28.6	29.7	31.5	30.3	27.9
	% CV	2.44	2.90	N/A	2.75	1.92	1.69	1.46	1.94
	Total Agreement with Positive Result	17/30 56.7%	26/30 86.7%	9/30 30.0%	30/30 100%	30/30 100%			30/30 100%
	95% CI	39.2% - 72.6%	70.3% - 94.7%	16.7% - 47.9%	88.7 – 100%	88.7 – 100%			88.7 – 100%
	Overall Average Ct Value	35.1	33.0	37.1	28.0	29.1	30.8	29.9	28.0
	Overall % CV	3.68	3.25	1.89	3.46	1.91	1.92	1.53	2.92

[a]Average Ct value for the Internal Control (IC)
*Agreement with Negative result

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
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Dr. Karen Harrington
Manager, Clinical Affairs
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W229 N1870 Westwood Drive
Waukesha, WI 53186

NOV 20 2009

Re: K091053
Trade/Device Name: ProParaflu+ ™ Assay
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OOU
Dated: October 14, 2009
Received: October 15, 2009

Dear Dr. Harrington:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not

limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (301) 594-3084. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address http://www.fda.gov/cdrh/dsma/dsmamain.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Indication for Use

510(k) Number (if known): k091053

Device Name: ProParaflu+™ Assay

Indication For Use:

The ProParaflu+ Assay is a multiplex Real Time RT-PCR *in vitro* diagnostic test for the qualitative detection and discrimination of Parainfluenza 1 Virus, Parainfluenza 2 Virus and Parainfluenza 3 Virus (HPIV-1, HPIV-2 and HPIV-3) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of respiratory tract infections. This assay targets the conserved regions of the Hemagglutinin-Neuraminidase (HN) gene of HPIV-1, HPIV-2 and HPIV-3, respectively. The detection and discrimination of HPIV-1, HPIV-2 and HPIV-3 nucleic acids from symptomatic patients aid in the diagnosis of human respiratory tract parainfluenza infections if used in conjunction with other clinical and laboratory findings. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b Viruses.

Negative test results are presumptive and should be confirmed by cell culture. Negative results do not preclude Parainfluenza 1, 2 or 3 virus infections and should not be used as the sole basis for treatment or other management decisions.

Prescription Use ___X___ (21 CFR Part 801 Subpart D) And/Or Over the Counter Use ____. (21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) k091053